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Oxidative Rancidity and Discoloration in Meat

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CONTENTS

I. Introduction

This review is concerned with two types of oxidative changes which occur in meat, namely, oxidation of the fat, resulting in rancidity, and

might be expected to affect rancidity, and special problems in the control of rancidity in meat arising from the fact that the fat is part of a heterogeneous system containing accelerators and inhibitors of rancidity.

1. The Nature of the Oxidative Process

Rancidity, at least as the term is used in meat products, results from the oxidative decomposition of unsaturated fats. The first step in this decomposition is the addition of oxygen at a carbon atom adjacent to an unsaturated carbon to form a hydroperoxide:

$$—CH_2.CH=CH.CH_2— + O_2 \rightarrow —CH(OOH)CH=CH.CH_2—$$

Although this reaction can occur in fatty acids having a single double bond, such as oleic, the methylene group between two double-bonded carbons is very much more susceptible to oxidative attack than carbons adjacent to a single double bond. Thus, linoleic acid, with one active methylene group, oxidizes ten to twelve times as rapidly as oleic acid. Linolenic acid, with two such labile carbons, oxidizes twice as fast as linoleic (Gunstone and Hilditch, 1945).

The hydroperoxides formed are intermediates in the oxidative process. They do not themselves contribute to the rancid odor, but they are unstable and break down to a great variety of decomposition products, some of which contribute to rancidity. Much recent work has been devoted to the isolation and identification of such decomposition products, but the literature is too voluminous to review here.

The course of the oxidation of any animal fat follows a typical pattern. A period of very limited oxygen uptake (the induction period) is followed by a phase of rapid oxidation (see Fig. 4). The induction period is due to the presence in animal fats of varying amounts of a natural antioxidant, *alpha* tocopherol. The activity of this and other antioxidants is discussed in Section V.

2. Species Variations in the Susceptibility of Animal Fats to Oxidation

As might be expected from the above discussion, the susceptibility of any natural fat to oxidative rancidity depends upon its degree of unsaturation (particularly with respect to fatty acids having more than one double bond) and upon its antioxidant content. It has long been known that certain fats, especially those of pork and poultry, are much more easily oxidized than other animal fats. Rancidity is not a pressing problem with beef or lamb, although Steinberg *et al.* (1949) showed that the palatability changes which take place on freezer storage of beef are correlated with the availability of oxygen, and Hines *et al.* (1951) found

deterioration in beef and lamb as well as pork in freezer storage to be due primarily to oxidation of the fat.

These species differences are probably to be attributed largely to the fatty acid composition. Chang and Watts (1952), in agreement with earlier studies, found pork and poultry fats to be much more unsaturated than beef or lamb fats. The linoleic acid content of the fat from beef and lamb cuts varied between 1 and 2% of the total triglycerides, whereas with pork it ranged from 7 to 10% and with poultry from 18 to 31%. Although there may also be species differences in the tocopherol content of the fat, such data as are now available do not indicate any clear-cut trend (Lange, 1950). The tocopherol content of all animal body fats is small, approximately 0.0005 to 0.003%, as compared to vegetable oils, most of which fall in the range 0.05 to 0.10%.

3. Influence of Rations on Fatty Acid Content of Animal Body Fats

Since the early work of Burr and Burr (1929, 1930) it has been recognized that animals are unable to synthesize fatty acids containing more than one double bond if none is supplied in the diet, although they apparently have the ability to utilize dietary fatty acids containing two or three double bonds to synthesize more highly unsaturated ones (Widmer and Holman, 1950). Thus, while saturated fatty acids and oleic acid can be synthesized from carbohydrates, fatty acids containing two, three, four, or more double bonds which appear in the body fat are derived from unsaturated fat in the diet. Certain species, including the hog, chicken, and turkey, tend to deposit fatty acids from the diet in their body fat to a much greater extent than other species.

Early work on the composition of body fats of hogs as related to diet and other factors is reviewed both by Lea (1939) and by Bailey (1951). When the oil or fat content of the feed is low, the pig synthesizes a firm fat, but when considerable portions of fat are fed, the pork fat reflects the composition of that fed. The linoleic acid content of hog fat could be varied from 2% (in low fat rations) to 32% (on soybean rations). Hogs having "firm" fat may be produced by feeding soybeans to young animals and then switching to a carbohydrate ration for the fattening period (Hostetler and Halverson, 1940), but it seems probable that this represents a dilution, not a replacement, of soft fat with hard. This ability to store unsaturated fatty acids extends also to linolenic acid. Beadle *et al.* (1948) found large amounts of trienoic acid in yellow hog fat and in fat of rats fed linseed oil.

Poultry fat presents the same general picture of wide variability in fatty acid composition. The early work of Cruickshank (1934) and several more recent studies (Kummerow *et al.*, 1948; Hite *et al.*, 1949;

Klose *et al.*, 1951) demonstrate conclusively the deposition of large amounts of unsaturated fatty acids from the ration in the body fats of chickens and turkeys. Linolenic acid, which is not normally present in animal fats to any appreciable extent, was deposited in poultry skin fat when present in the diet (Hite *et al.*, 1949). Since this fatty acid is normally present in such feed constituents as linseed meal, soybean meal, alfalfa, and whole wheat, it may be expected to be a variable constituent of poultry fats. Chang and Watts (1952) have also indicated the presence of highly unsaturated fatty acids with five and six double bonds in poultry fats, although it is not yet possible to say whether they are of dietary origin or are synthesized by the fowl from fatty acids with fewer double bonds.

The effects of these dietary variations in the fatty acid composition on the susceptibility of the fat and especially of the meat to rancidity are less clear-cut. One complicating factor is the fact that dietary sources of linoleic and linolenic acids are usually also good sources of tocopherol (Hove and Harris, 1951), and thus deposition of the antioxidant may to some extent counteract the greater unsaturation. In addition, when the fat is present in meat, variations in the aqueous medium surrounding the fat undoubtedly play an important part.

Shrewsbury *et al.* (1942) found in studies on the stability of hog fat that the soft fat (peanut-fed, iodine number 73–80) was consistently less stable than the hard (corn-fed, iodine number 58–60) both fresh and after frozen storage. However, there was more variation in the keeping time of hard fats from different lots of pork than in the keeping time of hard *versus* soft in any one lot. Peroxide values for fats extracted from the meats after freezer storage for 12 to 16 months were low and showed no significant differences between hard and soft fats. Brady *et al.* (1946) and Palmer *et al.* (1953) showed positive correlation between the softness of the fat and the susceptibility to rancidity of bacon and frozen ground pork, respectively.

Kummerow *et al.* (1948) found that the feeding of highly unsaturated fatty acids was detrimental to fat stability of eviscerated frozen turkeys, as determined both by peroxide values on the extracted skin fat and also by organoleptic tests on the cooked carcass. Klose *et al.* (1951) observed fishy flavors in roasted turkeys fed linseed oil as well as fish oils. The fishy flavors in this case were present in the fresh roasted turkeys as well as in those cooked after being stored in the freezer. Peroxide values of birds fed linseed oil increased very rapidly in freezer storage.

In addition to direct deposition of unsaturated fatty acids from the diet in the body fat of meat animals, recent work has indicated that various dietary supplements may influence the amount and composition

of body fats by an indirect effect on metabolic processes. Hite *et al.* (1949) fed supplements of ethanolamine and choline to poultry. The fat from the supplemented groups contained less of the 3 and 4 double-bond fatty acids and showed longer induction periods. Kummerow *et al.* (1949) were able to influence both the amount and also the composition of fat from rats on purified rations by a large number of supplements (gallates, tocopherol, butylhydroxyanisole, ascorbic acid, and especially pyridoxine). Sufficient data are not available to form any clear picture of the mode of action of these supplements as yet. With the exception of tocopherol they are not stored in the fat.

4. Deposition of Antioxidants in Animal Tissues

Barnes *et al.* (1943), Lundberg *et al.* (1944a), and Hanson *et al.* (1944) found that of a large number of antioxidants fed to rats only the tocopherols were stored in the rat adipose tissue, and the stability of the extracted rat fat (provided the fatty acid composition was not changed) depended entirely on the tocopherol content of the diet.

This early work on laboratory animals stimulated attempts to improve the keeping quality of rendered fat and meat by increasing the tocopherol in the fat and other tissues of meat animals through the feeding of tocopherols. Watts *et al.* (1946) fed tocopherol supplements to pigs, both on natural and purified rations. The total amounts fed (0.007 to 0.02% by weight of the pigs) gave some slight increase in stability of the rendered fat, but the magnitude of the effect was too small to be of great practical significance, although the larger amount is considerably more than could be achieved by any manipulation of natural rations.

The feeding of still larger amounts of tocopherol in relation to the weight of the experimental meat animals has resulted in more significant increases in tocopherol in the fat and various other tissues. Major and Watts (1948) improved the stability of rabbit fat in animals on purified rations by feeding or injecting tocopherol. Bratzler *et al.* (1950) showed a greatly increased tocopherol content of a number of hog tissues as well as

TABLE I

Rancidity Development and Tocopherol Storage in Turkey Tissues*†

Tocopherol total fed,‡ g.	Acceptability rating	Peroxides, m.eq./kg. Skin fat	Peroxides, m.eq./kg. Abdominal fat	Tocopherols, mg./kg. Heart	Tocopherols, mg./kg. Leg
4.2	Excellent	1	4.8	107	24
.4	Poor	1.8	5.9	60	15
0	Poor	2	6.7	52	13
E depleted	Poor	7.3	19.4	68	12

* Criddle and Morgan (1951).
† After 9 months freezer storage.
‡ Dose divided over 35 days just prior to slaughter.

fat from various parts of the body by feeding large amounts of tocopherol to animals on purified rations. Criddle and Morgan (1951) fed various levels of tocopherol to turkeys on natural rations and demonstrated not only increased tocopherol storage in various tissues at the higher levels but also improvement of stability of the fat on freezer storage. This in turn correlated with improved acceptability of the cooked meat after storage. A typical experiment is shown in Table I.

On the whole, the results of tocopherol feeding have been rather disappointing. It does not seem that very much improvement in the stability of meat fats can be achieved by manipulating ration components to secure naturally high levels of tocopherol. Although there is no doubt that additional tocopherol storage can be achieved in a variety of meat animals by feeding large tocopherol supplements and that such storage will improve the stability of the fat, this method is limited and economically wasteful. Only a very small fraction of the large doses of tocopherol fed are actually stored in the carcass.

5. Distribution of Fat in Meat as a Factor in Rancidification

In addition to these inherent characteristics of the fat itself, contact of the fat in meat with an aqueous solution containing surface-active substances, accelerators and inhibitors of rancidity, creates a very different situation from conditions which exist in a container of rendered lard. The author has noted on a number of occasions that the keeping time of fat rendered from pork tissues did not correlate with rancidity development in the ground meat. Schreiber *et al.* (1947) reported that the stability of fat, as measured by accelerated tests on the extracted fat from fresh birds, was not a good indication of the stability of poultry fat *in situ* during freezer storage.

The orientation of unsaturated fatty acids at an interface can have a profound effect on their oxidative behavior, even when the nonfat phase contains no known pro- or antioxidants. Table II shows the relative rates of oxidation of several fatty acids in bulk versus thin layers in contact

TABLE II

Relative Rates of Oxidation of Pure Fatty Acids under Various Conditions*

Distribution of fatty acids	Days to turn rancid at 24° C.†		
	Oleic	Linoleic	Linolenic
Exposed in bulk in watch glasses	13	1	<1
Thin layer absorbed on filter paper	5	2.3	1.6
Thin layer in contact with aqueous phase‡	5–8	3–4	2–3

* Lehmann and Watts (1952).

† As determined by half bleaching of dissolved carotene.

‡ Range of various experiments in which distilled water and buffers at pH 5.6 and 7.5 were employed as the aqueous phase.

with various aqueous solutions or absorbed on filter paper (relatively inert, but containing polar hydroxy groups). Whereas the oxidation of oleic acid is accelerated by its orientation at the interface, the more highly unsaturated fatty acids containing one or two active methylene groups oxidize less rapidly under these conditions. Apparently the active methylene groups are partially masked at such interfaces.

The effect of surface orientation can be demonstrated very clearly when the aqueous phase contains accelerators of rancidity. Hughes and Rideal (1933) spread monolayers of oleic acid on the surface of aqueous solutions of potassium permanganate. When the double bond touched the water, oxidation proceeded very rapidly. In more compressed films, the reactive double bonds were removed from the water surface and oxidation was greatly retarded. Eleostearic acid, containing three conjugated double bonds, lay flat on the surface and oxidized very rapidly. Haurowitz and Schwerin (1941) called attention to the importance of interfacial orientation of linoleic acid at the oil water interface in the hemin-catalyzed oxidation of this fatty acid.

Slight increases in tocopherol content which confer only limited improvement in stability of the rendered fat may have a much greater effect on the keeping quality of meat through synergistic activity with constituents of the muscle juice. Watts and Wong (1951), by increasing the tocopherol level of lard in contact with hemoglobin solution up to 0.005%, brought about a slight (50%) increase in keeping time. In contrast, when the hemoglobin solution also contained ascorbic acid, which acts synergistically with tocopherol, there was more than an eightfold increase in keeping time. A number of normal constituents of muscle juice may function in this way.

Much more information needs to be obtained on the effect of variations in muscle juice constituents on the stability of fat with which it is in contact in model systems which can be controlled, before it will be of much avail to attempt to change the media surrounding the fat in meat by dietary supplements.

6. Methods for Evaluating Oxidative Changes in Meat Fats

With the exception of organoleptic evaluations, all tests for rancidity in meat require the separation of the fat from other meat constituents. In most of the early studies this was accomplished by a preliminary drying of the meat followed by extraction of the fat with a fat solvent, usually in some form of continuous extraction apparatus. The disadvantages of this procedure have been recognized by many workers. Oxidation can take place during the drying process as well as during the subsequent lengthy extraction. Decomposition of preformed peroxides can also occur in hot

solvents (Watt *et al.*, 1949). More recent studies have avoided both the drying and the hot extraction and have reduced the time required for extraction to a few minutes by cold blending the sample and solvent in a Waring-type blender, preferably with a drying agent (Rockwood *et al.*, 1947; Watts and Peng, 1947b).

If the purpose is a rating of the degree to which the fat has undergone oxidation at the time of its extraction from the meat, as in meat storage studies, the fat can usually be analyzed for some product of oxidation without removing the solvent. The peroxide value remains the most widely used and generally satisfactory of such tests, in spite of the fact that peroxides are intermediates in the process of oxidative decomposition and are not themselves responsible for the rancid odor. Lea (1939) has reviewed the earlier work on this test. It is commonly carried out by estimating the amount of free iodine liberated by the oxidizing action of the fat peroxides on potassium iodide. A somewhat more sensitive test for peroxides was proposed by Lips *et al.* (1943) and independently by Sumner (1943) based on the oxidizing action of the fat peroxide on ferrous iron, followed by colorimetric estimation of the ferric iron as its colored complex with thiocyanate. Lea (1945) improved the method by excluding oxygen. Volz and Gortner (1947) have increased the sensitivity of the original iodometric procedure by carrying out the reaction in a single phase, using isopropanol as solvent.

The limitations inherent in the peroxide test as an objective method for rancidity have been discussed by Lea (1939, 1946a) and by Stansby (1941). The principal objection to its use is the fact that, because peroxides are intermediates, they are related to rancidity only as long as they are formed from the fresh oil at a rate greater than that of their decomposition to rancid products. If peroxides are breaking down more rapidly than they are forming, the peroxide number will decrease with increasing rancidity. This can occur, for example, when fats which have been stored at a low temperature are moved to a higher, or when fats in which peroxides have accumulated in the dark are exposed to light.

Mainly with the idea of overcoming these objections to the peroxide value as a measure of rancidity, various tests have been developed for decomposition products of oxidized fats. The widely used Kreis test, which depends upon the development of a red color when rancid fats are treated with phloroglucinol, has recently been shown to be given by malonic dialdehyde and other closely related constituents of oxidizing fats (Patton *et al.*, 1951). The test has been greatly improved in recent years by solution of all reactants in a single phase and colorimetric estimation of the color produced (Walters *et al.*, 1938; Pool and Prater, 1945; Watts and Major, 1946).

The reaction of oxidation products of linolenic acid with thiobarbituric acid to give an orange red color has been used by Abramson (1949), Wilbur *et al.* (1949), and others to follow unsaturated fat oxidation in various tissues. Patton *et al.* (1951) present evidence that this test, like the older Kreis test, also measures malonic dialdehyde.

In addition to the several tests for aldehydes of low and intermediate molecular weight, Lea (1939) and Pool and Klose (1951) have described a method for the estimation of monocarbonyl compounds in rancid foods based on their reaction with dinitrophenylhydrazine. Table III compares results of this test with peroxide values on turkey fats of varying degrees of oxidation.

TABLE III

Comparison of Monocarbonyl Compounds and Peroxides in Turkey Fat*

Individual birds	Storage time, months	Storage temperature, °F.	Monocarbonyl compounds, millimoles/kg.	Peroxide values millimoles/kg.
1	12	−30	0.05	0.0
2	12	−30	0.14	4.4
3	6	0	0.42	10.9
4	6	0	0.54	19.5
5	6	0	7.34	240.0

* Pool and Klose (1951).

Unfortunately, there is as yet no real evidence that any of these tests show any better correlation with organoleptic rancidity under changing conditions of storage than does the peroxide value. In fact, the Kreis test is even more sensitive than the peroxide value to changes in temperature and gives very high values with fats containing more highly unsaturated fatty acids. Comparisons of the peroxide test with one or more of the aldehyde tests and sometimes with organoleptic rancidity have been made on meat or poultry fats under a variety of storage conditions (White, 1941a; Watts and Major, 1946; Vail and Conrad, 1948; Mackey *et al.*, 1952).

Frequently it is desired to determine not the degree of oxidation which an extracted fat has already undergone but its susceptibility to oxidation under a standard set of conditions. This is the usual aim, for example, when it is desired to compare fats from animals on different rations or to compare the effects of antioxidants. This may be done by storing the fat under the desired set of conditions and following the course of oxidation with any of the tests described above. It may also be accomplished by manometric measurements of oxygen consumed (French *et al.*, 1935; Nagy *et al.*, 1944; Banks, 1944; Stirton *et al.*, 1945) or by following the rate of bleaching of fats to which a carotinoid pigment has been added,

since such pigments are oxidized very rapidly when fats have passed their induction period (Hove and Hove, 1944a; Lovern, 1946; Watts and Peng, 1947a; Bickoff *et al.*, 1952).

Most of the studies on fat stability have been carried out with the dry fats. Of more direct application to the control of rancidity in meats are artificial systems in which meat fat is brought into contact with an aqueous phase. Lea (1937), used emulsions of muscle juice in lard and in some cases glass slides coated with lard and then immersed in thin layers of the aqueous solution. Scarborough and Watts (1949) and Lehmann and Watts (1951) have described a method for achieving contact by bringing together filter papers saturated with the fat and aqueous phases, respectively. Carotene dissolved in the fat serves as a convenient indicator of the onset of rancidity. The method is crude but does allow the rapid evaluation of antioxidants, meat constituents, or additives under conditions which more closely resemble those in meat. The Warburg manometric technique has also been used for investigation of aqueous fat systems (Banks, 1944), but in this case the fat-water interface is constantly changed by the shaking.

III. Oxidative Discolorations

1. Normal Pigments of Fresh Meat

The typical reddish colorations of both fresh and cured meats are contributed by myoglobin and hemoglobin and their derivatives. Although more than 90% of the pigment present in fresh meats is myoglobin rather than hemoglobin (Shenk *et al.* 1934), a much greater volume of work has been done on the latter pigment, since it can be obtained easily in large quantities by laking washed red blood corpuscles. Myoglobin is much more difficult to obtain in a sufficient state of purity. Reliable information concerning its properties awaited its crystallization by Theorell (1932). Millikan (1939), in a review which summarizes the existing information up to that time, compares the muscle and blood pigments in a number of important properties. In structure both have the same prosthetic group, *i.e.*, heme, an iron porphyrin. However, the globin to which the heme is attached is different in the two pigments and, whereas myoglobin consists of a single heme compound (molecular weight 17,500), the hemoglobin molecule contains four hemes, each associated with globin. The molecular weight of hemoglobin is thus 68,000.

Both pigments combine reversibly with oxygen, carbon monoxide, and nitric oxide to form bright red oxyhemo (or myo) globin, carbon monoxide hemo (or myo) globin, and nitric oxide hemo (or myo) globin, respectively. In all of these compounds the iron remains in the ferrous

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form and the equilibrium is governed in each case by the partial pressure of the respective gas. Both hemoglobin and myoglobin may lose an electron, becoming oxidized to the corresponding brown "met" pigments. Both may undergo changes of the porphyrin ring to give green or grey decomposition products (see Section III, 2).

Whereas the two pigments undergo the same qualitative reactions, there are several important quantitative differences between them which should be recognized in applying data obtained on the blood pigment to meat. Myoglobin has a much greater affinity for oxygen than does hemoglobin, but a much lower affinity for carbon monoxide (and probably also for nitric oxide, although data seem to be lacking). Myoglobin oxidizes to the brown ferric compound fourteen to sixteen times as rapidly as hemoglobin when exposed to atmospheric oxygen (Kiese and Kaeske, 1942). The displacement of the absorption maxima of myoglobin toward longer wavelengths and its greater stability in alkaline solutions are the bases for the spectrophotometric differentiation of the two pigments in their mixtures (Shenk *et al.*, 1934; Watson, 1935; Fanelli, 1949).

The color of fresh meat is typically the bright red of the oxygenated heme pigments at surfaces exposed to air and the purplish red of the reduced pigments in the interior. These variations in color, as a function of oxygen tension, are normal and characteristic of fresh meat; nevertheless the purplish red color, especially in ground meats such as hamburger, is often objectionable to consumers and is not distinguished from the dulling or browning due to methemoglobin formation.

Coleman and Steffen (1949), in a patent assigned to Armour and Company, propose the use of niacin to bring about a uniform bright red color throughout fresh meats, owing to the formation of "a new pigment reaction product which is bright red in color." No information is available on the nature of this compound. The amount of niacin recommended (0.3 g. per pound of meat) is much larger than the amounts normally occurring in meat. Hopkins *et al.* (1950) from the same laboratory have patented a process for maintaining uniform red color in ground meats by introducing oxygen mechanically. This is accomplished by freezing the ground meat, breaking it into small pieces while frozen, and subjecting the pieces to pressure sufficient to shape them into patties but not sufficient to drive out the entrapped air.

2. Oxidation Products of Heme Pigments

Two types of oxidative changes are chiefly responsible for the abnormal brown, grey, and green discoloration of meat. One involves the oxidation of the ferrous iron in the heme compound to the ferric condition; the second is a direct attack by oxygen on the porphyrin ring.

The most commonly encountered type of discoloration is that of the brown oxidation products, methemoglobin and metmyoglobin, formed from the normal blood and muscle pigments by oxidation of the iron to the ferric state (Brooks, 1929, 1938). Various aspects of this oxidation are discussed at length in several recent reviews (Granick and Gilder, 1947; Theorell, 1947; Wyman, 1948; Granick, 1949; Lemberg and Legge, 1949; Haurowitz, 1950).

The question as to why the heme pigments sometimes combine reversibly with atmospheric oxygen to form the bright red oxygenated pigments of normal meat and at other times become oxidized to the brown ferric compounds is not clear. The ability of hemoglobin and myoglobin to combine reversibly with oxygen depends upon their specific protein linkage with native globin. Other heme proteins of tissues (peroxidase, catalase) do not possess this ability even though the iron porphyrin portions of the molecule are identical.

Denaturation of the globin destroys the ability of hemoglobin or myoglobin to combine reversibly with oxygen and greatly increases the susceptibility of these pigments to true oxidation. Part of the oxygen liberated by the denaturation of oxyhemoglobin oxidizes the iron; part probably attacks globin itself (Holden, 1936). The ferrous, denatured globin hemochromogen formed by denaturation of hemoglobin under reducing conditions is much more susceptible to oxidation than is hemoglobin itself. The oxidation potential of the ferrous-ferric hemochromogen system is -0.098V at pH 7.06, whereas the corresponding potential of the hemoglobin-methemoglobin system is between $+0.144$ and $+0.152$ (Lemberg and Legge, 1949).

It is probable that even partial and reversible denaturation of the globin, which may not be accompanied by coagulation, can accelerate the rate of oxidation. The many agents, not themselves direct oxidizing agents, which are known to accelerate oxidation of the iron of oxyhemoglobin, may act indirectly by disturbing, at least temporarily, the bonds between heme and globin. Granick (1949) has pointed out that the drugs which bring about methemoglobin formation are those which denature globin. Factors which accelerate oxidation of fresh meat pigments, such as heat, freezing, acid, salt, ultraviolet light, and certain metals, are known to denature globin.

Irrespective of the mechanism of methemoglobin formation, it is known that this pigment is continually formed and reduced in the blood of living animals (Cox and Wendel, 1942). The reducing mechanisms normally operative in the living animal have been studied extensively, usually with the objective either of combating pathological conditions of methemoglobinemia or of preserving whole blood for intravenous

injection. Much of this work has been reviewed by Granick (1949), Lemberg and Legge (1949), and Bodansky (1951).

Reduction of methemoglobin in red cells is dependent upon the glycolytic system of the cells. Reduced diphosphopyridine nucleotide (DPN-H_2) produced during glycolysis can reduce methemoglobin, but not directly. Electron mediators, normally flavines, are essential. Several additives have proved effective in accelerating methemoglobin reduction through this system and have been suggested for use under varying conditions. Methylene blue catalyzes reduction of methemoglobin by DPN-H_2 (Gutman *et al.*, 1947). Various substrates, including glucose and other hexoses, lactate, fumerate, malate, citrate, etc., can, under some conditions, accelerate methemoglobin reduction, presumably by increasing the rate of reduction of DPN (Spicer *et al.*, 1949; Pennell and Smith, 1949; Gibson, 1948; Gutman *et al.*, 1947). The addition of nicotinamide has been useful in preventing hydrolysis of DPN (Gutman *et al.*, 1947).

All of the above work has been done on blood or its derivatives. Similar studies on the formation and reduction of metmyoglobin in tissues are for the most part lacking. Jensen (1935) patented the addition to meat (by arterial pumping just after slaughter) of various organic acids or their salts, with the idea of increasing reducing conditions within the meat through action of cellular dehydrogenases on the added acids.

Methemoglobin may also be reduced directly by various reducing agents such as sodium sulfite (Jensen and Urbain, 1936a), titanous citrate (Ramsay, 1944), dithionite (Lemberg and Legge, 1949), glyceraldehyde (Kiese, 1943), cysteine (Kiese, 1943), and ascorbic acid (Gibson, 1943; Kiese, 1943). Only the latter has demonstrated usefulness in meats (see Section V, 3).

In addition to brown and grey discolorations due to the formation of methemoglobin, very objectionable greenish pigments may appear in meats. Lemberg and Legge (1949) have reviewed in detail various transformations of the heme pigments which result in green compounds. All of these involve an attack on the porphyrin ring, usually at the α methene bridge. The essential change seems to be elimination of the double bond at this point, thus interrupting the series of conjugated double bonds which comprises the porphyrin ring and destroying the resonance structure. It is not essential that the ring be ruptured at this point; in fact, there is good evidence that at least two green compounds, choleglobin (Lemberg and Legge, 1950) and sulfhemoglobin, retain their closed porphyrin rings. Further oxidation, involving opening of the ring with splitting out of the α methene carbon atom, can then occur (verdohemes). Figure 1 shows suggested structures of choleglobin and verdoheme. Iron

is easily removed from the opened ring to give the bile pigment biliverdin. Other methene bridges may be attacked in the same way, giving rise to three, two, and one pyrrole fragments which range in color from yellow to colorless.

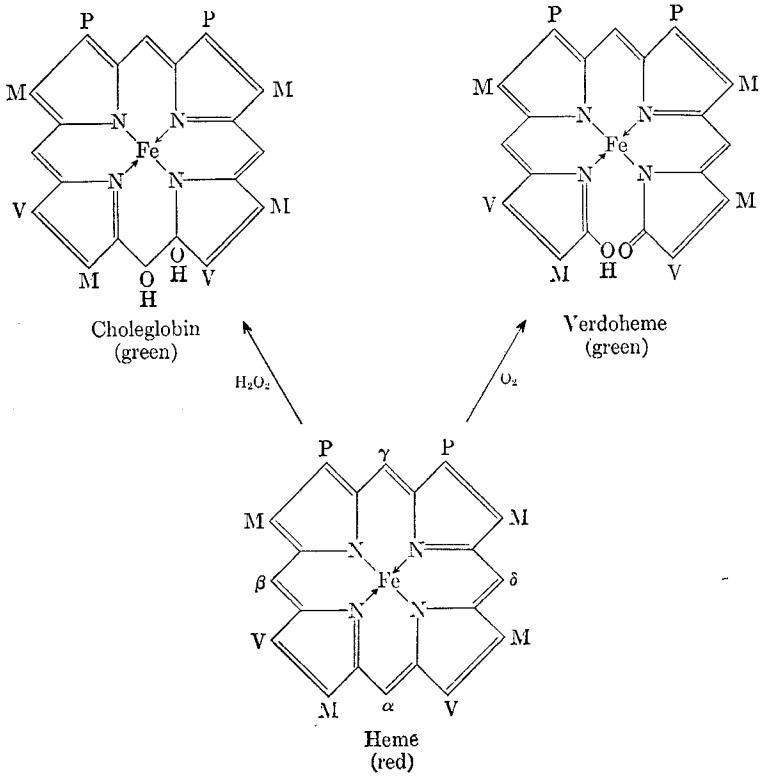

FIG. 1. The formation of green porphyrins from hemoglobin. The side chains on the porphyrin ring are abbreviated, *i.e.*, M = methyl, P = propionic acid, V = vinyl. In both green porphyrins the conjugated double-bond system of the porphyrin ring is interrupted at the α methene bridge.

Such attacks on the porphyrin ring may occur in meat under a variety of conditions. Sulfhemoglobin (formed directly upon addition of sulfide or thiosulfates in the presence of oxygen) has been attributed to hydrogen-sulfide bacteria, discussed by Jensen (1945). Any reaction which will produce hydrogen peroxide under conditions where it is not readily decomposed by catalase (as in cured meats where catalase is absent) results in such rapid and intense greening that the reaction has been proposed as a delicate test for heme pigments by Jensen and Urbain (1936b). Here again, peroxide-forming bacteria have been implicated

(Jensen and Urbain, 1936a; Jensen, 1945; Niven *et al.*, 1949; Niven, 1951). Methemoglobin and metmyoglobin, as well as hemoglobin and myoglobin, form unstable addition compounds with hydrogen peroxide, which then decompose with destruction of the heme nucleus (Keilin and Hartree, 1950).

A similar greening reaction occurs when various hydrogen donors are brought into contact with oxyhemoglobin or oxymyoglobin at physiological temperatures. This coupled oxidation has been studied most extensively with ascorbic acid and hemoglobin (Lemberg *et al.*, 1939, 1941; Kiese and Kaeske, 1942; Foulkes and Lemberg, 1949; Takeya, 1949; Kikuchi, 1950; Watts and Lehmann, 1952a). In this case the greening is caused by the formation of an unstable hydrogen peroxide derivative of hemoglobin (Lemberg *et al.*, 1939).

3. The Pigments of Cured Meats; Their Oxidation

The general chemistry of the meat-curing process is well covered by Urbain (1951) and the part played by bacteria in this process by Jensen (1945). Jensen (1949) has also described the curing process and various types of cured meat products in nontechnical language.

The cured meat pigment is nitric oxide hemochromogen, formed by the heat denaturation of nitric oxide hemo (or myo) globin (Haldane, 1901). The latter compound is a ferrous heme derivative similar to oxymyoglobin except that oxygen is replaced by nitric oxide. Nitric oxide hemoglobin can be prepared directly by passing nitric oxide gas through hemoglobin solutions under anaerobic conditions (Keilin and Hartree, 1937; Urbain and Jensen, 1940) or more conveniently by the addition to hemoglobin solutions of a strong reducing agent and a nitrite salt (Jensen and Urbain, 1936a). The pigment is bright red with an absorption spectrum very similar to that of oxyhemoglobin (Fig. 5).

While there is no doubt that nitric oxide can combine directly with reduced myoglobin to form nitric oxide myoglobin, which retains its redness on heat denaturation, the sequence of events leading to the formation of the cured meat pigment in meats is much less clear. The active curing ingredient, nitrite, reacts with oxyhemoglobin to form methemoglobin (Greenberg *et al.*, 1943) according to the reaction:

$$NO_2^- + 2HbO_2 \rightarrow NO_3^- + Hb_2O + O_2$$

This reaction is very rapid in the acid range of normal meat. To the extent to which the meat pigment is in the oxygenated form when brought into contact with curing salts, this is the first reaction which occurs. Even in the absence of oxygen, nitrite reacts with hemoglobin to give one molecule of nitric oxide hemoglobin and one of methemoglobin, if substances

capable of reducing both methemoglobin and nitrite are not present (Brooks, 1938).

In comminuted meats such as frankfurters, the entire mass turns grey upon mixing with the curing salts. Color fixation (normal pink color of cured meat) takes place during the gradual heating in the smokehouse and the subsequent cooking. The heat treatment given most cured meat products is an important factor in developing this color. Winkler and Hopkins (1940) made objective measurements on a photoelectric comparator of the "total brightness" of bacon, *i.e.*, intensity of the reflected light in samples heated to various temperatures for different lengths of time (Table IV).

TABLE IV

Total Brightness (Average) of Bacon Samples at Conclusion of Heat Treatment*

Duration of heat treatment, hours	Temperature, °C. 20	40	50	60	70	80
5	121	120	126	154	163	168
10	115	115	134	155	163	158
20	113	128	139	152	157	143
40	—	143	158	161	169	128
Mean	116	126	139	156	163	149

* Winkler and Hopkins (1940).

Current theories of the chemistry of meat curing assume that reduction of metmyoglobin is brought about by cellular reducing systems during early stages of the heating. The reduced myoglobin then combines with nitric oxide (similarly formed by reduction of nitrite) and the resulting nitric oxide myoglobin is converted by further heating to the corresponding red (pink) denatured globin hemochromogen. The latter is believed to be less subject to oxidation than the undenatured pigment because reactivity is reduced by loss of solubility on coagulation.

It seems doubtful that this is an adequate explanation of the observed facts. Not only heat but many other factors known to accelerate globin denaturation, such as freezing, salt, acid, and certain metals, also accelerate the formation of nitric oxide myoglobin or nitric oxide denatured globin hemochromogen. This acceleration occurs not only in meat but also in relatively pure methemoglobin preparations in the presence of nitrite and a reducing agent such as ascorbic acid, and the acceleration is very evident even at such early stages in the denaturation of the globin that no coagulation has occurred (the solution remaining optically clear). Thus, the same agents which bring about oxidation of oxyhemoglobin to brown ferric pigments, also bring about formation of red ferrous nitric oxide derivatives from methemoglobin in the presence of curing salts

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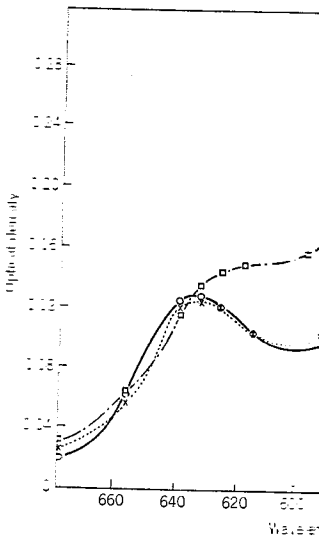

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Further investigation of the forma
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(Watts and Lehmann, 1952a, b). With respect to these factors, the conditions necessary to develop and retain cured meat color are exactly the reverse of those necessary to protect fresh meat color.

The mechanism by which protein denaturing agents are able to effect reduction of the ferric iron in the presence of nitrite (or nitric oxide) is obscure. It is probable that an intermediate is involved. Keilin and Hartree (1937) demonstrated that nitric oxide combined not only with

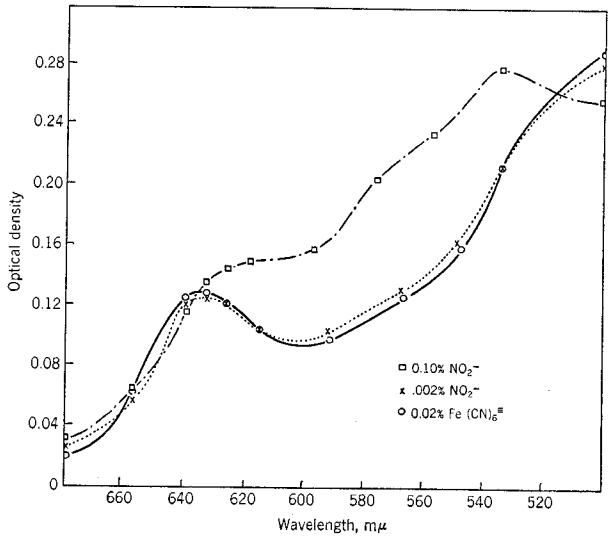

FIG. 2. Absorption spectra of compounds formed upon addition of nitrite to oxyhemoglobin (Watts and Faulkner, 1953). Ferricyanide and the lower concentration of nitrite produce the typical absorption spectrum of methemoglobin. The curve obtained with the higher concentration of nitrite probably represents a mixture of methemoglobin and methemoglobin nitrite.

reduced hemoglobin to give nitric oxide hemoglobin but also with methemoglobin to give an unstable nitric oxide methemoglobin which undergoes a slow autoreduction to nitric oxide hemoglobin. Barnard (1937) has presented evidence for the formation of a methemoglobin nitrite to explain changes from a brown to a red color in solutions containing a high ratio of nitrite to hemoglobin. Further evidence for this compound is presented in Fig. 2. At a molar nitrite-to-hemoglobin ratio of 5 to 1, the solution is brown and the absorption spectrum is practically identical with that of methemoglobin prepared by the addition of ferricyanide. Upon the addition of a 50-to-1 ratio of nitrite, the solution is much redder and has a single peak at 540 mμ.

Further investigation of the formation, stability to oxidation, and other properties of compounds of methemoglobin with oxides of nitrogen

should be profitable in the control not only of the normal curing process but also of such abnormal conditions as "nitrite burn."

The oxidation products of nitric oxide hemoglobin are the same as those from hemoglobin. The transformation of nitric oxide hemoglobin

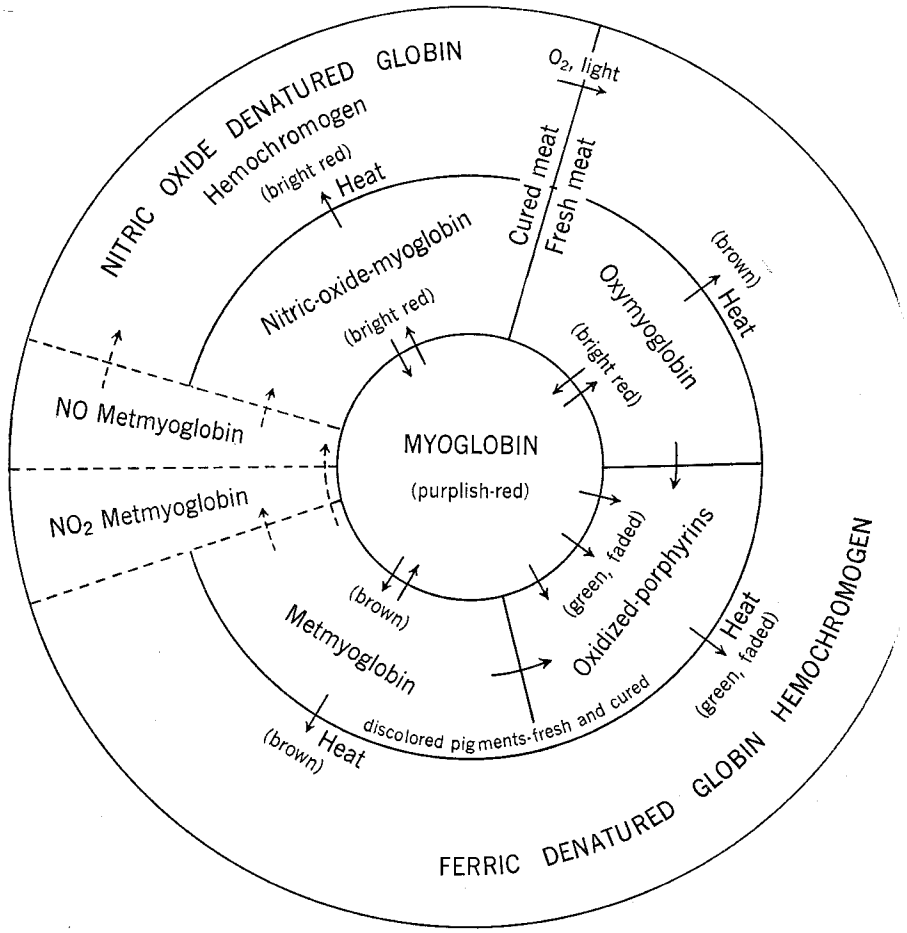

FIG. 3. Derivatives of myoglobin of importance in meats. In the outer circle are represented the insoluble hemochromogens obtained by coagulation. Only in the case of the cured meat pigment is the denatured compound red. Dotted portions represent possible intermediates between metmyoglobin and the cured meat pigment.

to methemoglobin in the presence of oxygen is very rapid, even though nitric oxide hemoglobin is stable indefinitely in the absence of oxygen and does not lose its combined nitric oxide when subjected to a high vacuum (Urbain and Jensen, 1940).

Nitric oxide hemoglobin and the corresponding denatured hemochromogen are highly subject to attack of the porphyrin ring by preformed hydrogen peroxide. The greater susceptibility of the cured meat as compared to fresh meat pigments to greening by hydrogen peroxide (Jensen and Urbain, 1936a) is at least partly to be explained by the catalase activity of blood and fresh meat and the destruction of this enzyme in formation of the cured pigment. On the other hand, the cured meat pigment is not greened by hydrogen donors such as ascorbic acid (Watts and Lehmann, 1952a).

Figure 3 is a schematic representation of the interrelationships of the pigments of fresh and cured meats and their oxidation products.

4. Methods for Investigation of Color Changes in Meat

Spectrophotometric methods for the analysis of hemoglobin and various derivatives in solutions have been widely employed in routine clinical work as well as in research studies. The literature is voluminous and will not be reviewed here. Similar methods are applicable to studies of myoglobin in muscle extracts. Bowen (1949) published absorption spectra and extinction coefficients of myoglobin and a number of its derivatives in wavelengths ranging from 1000 to 450 mμ. These apply, of course, only to clear solutions of the pigments in question. Husaini *et al.* (1950) give a simple procedure for preparing clear muscle extracts for myoglobin determination.

Winkler (1939a) and Winkler *et al.* (1940) described an objective method for following color changes at meat surfaces, using a photoelectric color comparator. Reflected light in the red, green, and blue regions of the spectrum, defined by standard colored filters, was measured photoelectrically and expressed as per cent of the amount scattered in the same spectral regions from a standard white surface under the same light intensity. Total reflection, obtained by adding values from all three regions, was used as a measure of the brightness of the meat surface. A number of commercial instruments, similar in principle to that described by Winkler, are now available.

Using a reflectance attachment to the Beckman spectrophotometer, Ramsbottom *et al.* (1951) followed fading of the surfaces of cured meats by measuring the ratio of light reflected at 650 mμ to that at 570 mμ. Reflectance curves are needed over the entire visible region of the spectrum, from meat surfaces on which the pigments have been converted by suitable means to known forms, such as nitric oxide myoglobin and metmyoglobin, before any of the above methods can be widely applied in meat studies.

IV. The Coupled Oxidation of Hemoglobin and Unsaturated Fats

In addition to the independent oxidation of unsaturated fats and of the heme pigments in meat, there is also a reaction between the two which brings about their mutual oxidation, accelerating both rancidity and color loss.

Robinson (1924) first described the catalytic effect of the hemes on the oxygen uptake of linseed oil. Hemoglobin, methemoglobin, and hemin all had about the same accelerating effect in equivalent concentrations. Inorganic iron had a relatively slight effect and the porphyrins, after removal of iron, none at all.

The more rapid oxidation of the fat is accompanied by concomitant oxidation of the hemoglobin. Niell and Hastings (1925) used linseed oil to accelerate oxidation of hemoglobin to methemoglobin. Haurowitz *et al.* (1941) demonstrated the destruction of the porphyrin during its prolonged reaction with unsaturated fat. The color faded and inorganic iron was released, but neither porphyrins nor bile pigments could be identified as cleavage products. The reaction was limited to fatty acids more unsaturated than oleic. The following figures were obtained after hemoglobin and unsaturated fatty acids were shaken in a Warburg for 2½ hours:

Fatty Acid	O_2 Absorption, cu. mm.	Catalyst Destroyed, %
linoleic	351	65
oleic	17	8

The mechanism of the reaction has not been extensively studied. Barron and Lyman (1938) attributed the catalytic effect to initiation of new reaction chains by the heme compounds. Banks (1944) suggests that the active catalyst is a combination of heme and fat peroxides. The reaction takes place only in heterogenous systems (Haurowitz and Schwerin, 1941; Lovern, 1946); if heme compounds and fatty acids are dissolved in the same solvent the rapid oxidation does not occur. It may be that the much greater unit efficiency of the iron in hemoglobin as compared to inorganic iron as an electron transfer medium for the oxidation of unsaturated fats is due entirely to concentration and orientation of hemoglobin at the interface, in contact with the unsaturated fat (Harper, 1953).

Since hemoglobin and myoglobin are brought into intimate contact with fat in meats, this coupled reaction might be expected to contribute both to rancidity and discoloration. Extracts from pork tissue, both muscle and fat, have been found by a number of workers to accelerate fat

…Lea, 1937), who first obser…
…and Peng (1947a) accounted fo…
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TABLE …

…Effect of pH on Rancidity Developme…

Lactic acid added %	pH of raw meat†
None	6.5
0.031	6.4
0.103	6.1
0.206	5.6
0.617	4.8

…and Peng, 1947b).
† …samples was usually 0.1 to 0.2 higher …

oxidation. Lea (1937), who first observed this, attributed it to a lipoxidase. Watts and Peng (1947a) accounted for the accelerating effect of extracts from pork muscle by the myoglobin present. On the other hand, Reiser (1949) believed the catalytic effect of aqueous extracts of bacon adipose tissue on fat oxidation to be due to an enzyme. He based his conclusions on the heat lability of the catalyst and the fact that after removal of heme pigments catalysis still occurred. Chang and Watts (1949) ascribe this loss of activity on heating to coagulation of the hemoglobin. Tappel (1952) came to the conclusion that the catalyst in both muscle and fat is a heme pigment, not a lipoxidase of the type known to occur in plants, since the catalytic effect here is apparent in heterogenous systems only, whereas lipoxidase is even more active in homogeneous solutions.

The importance of this reaction in contributing to the oxidative changes which take place in meat is difficult to evaluate. It is certainly a contributing factor in the deterioration of ground meats preserved by freezing (Watts and Peng, 1947b), but may be of less importance in large cuts, frozen whole (Watts *et al.*, 1948). Klose *et al.* (1950) found that turkey dark meat turned rancid in storage much more rapidly than the white meat.

Lea (1937) and Watts and Peng (1947a) observed that the rancidifying effect of muscle extracts fell off with increasing pH (within the range of normal meat). Experiments on frozen ground pork adjusted to different pH values (Watts and Peng, 1947b) have demonstrated the same close correlation of rancidity and pH. Fading of the color accompanies rancidification. pH ranks with tocopherol content and fatty acid make-up of the fat as a major cause of variation in freezer storage life of meat from different carcasses.

Heating hemoglobin solutions, muscle extracts, or meat enough to coagulate the hemoglobin or myoglobin eliminates their catalytic effect on fat oxidation. The heating does not destroy the iron porphyrin which

TABLE V

The Effect of pH on Rancidity Development in Raw and Precooked Pork Sausage*

Lactic acid added %	pH of raw meat†	Peroxide value after 4.5 months storage, millimoles/kg. Raw	Cooked
None	6.5	2.0	3.3
0.031	6.4	1.6	3.7
0.103	6.1	5.9	2.9
0.206	5.6	16.9	3.6
0.617	4.8	25.2	4.7

* Watts and Peng (1947b).
† pH of cooked samples was usually 0.1 to 0.2 higher than raw.

is the active catalyst, but presumably inactivates it by rendering it insoluble as the globin is coagulated. Changes in pH no longer affect the rate of rancidification of meat after cooking (Table V).

As would be expected considering the general effectiveness of all iron porphyrins so far tried as catalysts of fat oxidation, nitric oxide hemoglobin accelerates rancidity to the same extent as hemoglobin at the same concentration (Chang and Watts, 1949). Tappel (1952) found that extracts from cured pork as well as from raw pork accelerated oxidation of linoleic acid. No information is available on color changes in nitric oxide hemoglobin during the course of the catalytic process.

V. Antioxidants

The development of new chemicals for the protection of fats from oxidative changes has progressed at a very rapid rate during the past decade. Hilditch (1944) has reviewed some of the British work on stabilization of dried foods, including meats, with antioxidants. Lundberg (1947) made a survey of the antioxidants proposed for use in foods at that time and Riemenschneider (1947) reviewed briefly the activity of antioxidants of interest to cereal chemists. Unfortunately, there does not seem to be a recent comprehensive review of the subject. Space limitations will not permit more than a brief résumé here, directed particularly at the possible usefulness of these compounds in meat.

1. Classification and Mode of Action of Fat Antioxidants

Most compounds which have a direct antioxidant effect on pure unsaturated fatty acids or their glycerides are phenolic substances. The antioxidants of this type which have been approved by the Bureau of Animal Industry for use in lard are: the naturally occurring tocopherols 0.03% (Olcott and Emerson, 1937; Golumbic, 1941, 1943; Hove and Hove, 1944b); gum guaiac 0.1% (Newton and Grettie, 1933; Doegey, 1943; Black, 1950); nordihydroguaiaretic acid (NDGA) 0.01% (Lundberg *et al.*, 1944b), propyl gallate 0.01% (Golumbic, 1942; Boehm and Williams, 1943) and butylhydroxyanisole (BHA) 0.02% (Kraybill *et al.*, 1949; Dugan *et al.*, 1951; Rosenwald and Chenicek, 1951).

Compounds of this type extend the induction period of oxidizing fats, presumably by absorbing the activating energy of fat peroxides, thus breaking chain reactions which might otherwise extend to several hundreds or even thousands of fat molecules. The antioxidants are themselves oxidized during this process (Filer *et al.*, 1944; Lundberg *et al.*, 1947; Mahon and Chapman, 1953). Figure 4, taken from the data of Mahon and and Chapman, illustrates the increase in the induction period of a sample

of lard brought about by an
gallate and the fate of the

In addition to the phenolic
compounds of widely differen
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keeping qualities of animal fats
and which are therefore termed
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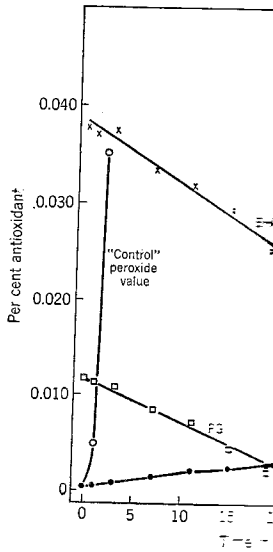

Fig. 4. Peroxide values and antioxida
man, 1953). The control contained no a
contained 0.04% butylhydroxyanisole
.08% citric acid. Stored at 61° C.

.005% (Lindsey and Maxwell, 19
1934, 1935; Kraybill and Beadle, 19
s esters (O'Leary, 1946) and le
Olcott and Mattill, 1936). Many oth
have synergistic activity, including
amino acids (Clausen *et al.*, 1947
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The mode of action of this var
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of lard brought about by an antioxidant mixture of BHA and propyl gallate and the fate of the antioxidants during this period.

In addition to the phenolic antioxidants, there are a large number of compounds of widely different chemical composition which have no protective effect when added to pure triglycerides but which enhance the keeping qualities of animal fats if added along with a phenolic antioxidant and which are therefore termed "synergistic" antioxidants. Members of this group which have been approved as additives to lard are citric acid

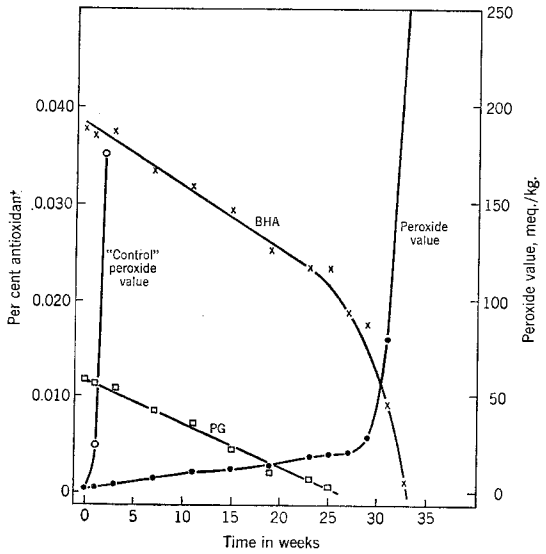

FIG. 4. Peroxide values and antioxidant destruction in lard (Mahon and Chapman, 1953). The control contained no added antioxidant. The experimental sample contained 0.04% butylhydroxyanisole (BHA), 0.012% propyl gallate (PG), and 0.008% citric acid. Stored at 61° C.

0.005% (Lindsey and Maxwell, 1949); phosphoric acid 0.005% (Eckey, 1934, 1935; Kraybill and Beadle, 1948); thiodipropionic acid 0.01% and its esters (O'Leary, 1946) and lecithin in any amount (Evans, 1935; Olcott and Mattill, 1936). Many other compounds have been reported to have synergistic activity, including normal constituents of meat such as amino acids (Clausen *et al.*, 1947), ascorbic acid (Golumbic and Mattill, 1941; Calkins and Matthill, 1944), nicotinic acid (Taub and Simone, 1947), and para-aminobenzoic acid (Norris, 1949).

The mode of action of this varied group of compounds is not well understood and is probably not the same for all synergists. Many have the ability of combining with metals which would otherwise accelerate oxidation. Some, such as ascorbic acid, may reduce the oxidized forms of

the primary antioxidant (Golumbic and Mattill, 1941). Others, which are not themselves reducing agents, *i.e.* phosphoric acid and various organic acids, may form fat-soluble complexes with primary phenolic antioxidants. The complexes may diffuse into the fat and there react with activated fat molecules, absorbing the excess energy and so breaking the chain reaction (Calkins, 1947).

2. Application to Meats

While all of the compounds approved for use in lard have been thoroughly tested for toxicity and have established their usefulness in protecting the rendered fat, none has been given official sanction as an additive to meat. The problem of protecting meats is more complicated than that of protecting rendered fats. It is essential that the antioxidant chosen should protect the fat when it is in contact with muscle juice. Further, the antioxidant must be capable of uniform distribution in the meat.

A number of phenolic antioxidants retarded the oxidation of unsaturated fats in contact with hemoglobin solutions (Barron and Lyman, 1938; Banks, 1944; Chang and Watts, 1949). In contrast, the water-soluble synergistic antioxidants, with the exception of ascorbic acid, had no effect on the hemoglobin-catalyzed oxidation, even when the fat contained added tocopherol, although many were active when the hemoglobin was coagulated by heat and so might be expected to be effective in cooked meats. These studies were made on artificial systems where tocopherol or other phenolic antioxidants could be introduced directly into the fat and the synergists into the aqueous phase.

The problem of getting the antioxidant into tissue fat has not been adequately solved. The most effective phenolic inhibitors are practically insoluble in water. Attempts at utilizing them in meat generally involve either their solution in a fat which is then applied to the surface of meat cuts or their dispersion with various carriers and emulsifying agents in curing brines, cooking waters, and comminuted meats.

For example, Smith *et al.* (1945) and Brady *et al.* (1946) lengthened the induction period of bacon slices by applying vegetable oils and phenolic antioxidants to the surface. Davis and Bywaters (1951) prolonged the freezer storage life of eviscerated broilers by dipping or spraying them with a solution of melted vegetable fat containing NDGA, ascorbic acid, and a vegetable gel. Fonyo (1950) patented a treatment for indigenous tissue fats which consists of NDGA emulsified in sorbitan derivatives of various fatty acids or polyethylene glycols. The solution is then diluted with water or brine. Komarik and Hall (1951) patented an accelerated curing process for bacon which includes a preliminary soaking in an

aqueous bath in which various anti
issued to Cornwell (1951) describes
which antioxidants as well as bacteri
dispersed in a 2 to 10% solution of
1952) protected turkeys in freezer sto
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Cooked meats preserved by freezi
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3. Use of Antioxidants

The fact that an antioxidant may
from rancidity does not mean that it wi
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will have a beneficial effect on color. T
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Wiesman and Ziemba (1946) found that
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no information was given on the effect of

The only antioxidant which has sho
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reduces methemoglobin and metmyoglob
olor-stabilizing agent in fresh ground m
use in fresh meats seems to be limited
higher temperatures or in the freezer it

aqueous bath in which various antioxidants are dispersed. A patent issued to Cornwell (1951) describes a protective coating for hams in which antioxidants as well as bactericidal and fungicidal compounds are dispersed in a 2 to 10% solution of hydroxyethylcellulose. Klose *et al.* (1952) protected turkeys in freezer storage by coating them with various antioxidant mixtures in propylene glycol and gelatin.

Cooked meats preserved by freezing or drying respond well to antioxidant treatment. Lea (1944) extended the life of dehydrated pork by incorporating ethyl gallate and gum guaiac into it before drying at concentrations of 0.02–0.1%. The antioxidants were dissolved in alcohol, which in turn was mixed with hot fat and added to the cooked meat. Morgan and Watts (1948) made use of the natural antioxidants present in soybean flour as well as added gum guaiac, tocopherol, and ascorbic acid to protect dehydrated pork scrapple. Lineweaver *et al.* (1952) added a commercial antioxidant preparation consisting of 20% BHA, 6% propyl gallate, and 4% citric acid in 70% propylene glycol to the water in which turkeys were cooked before preservation as the frozen creamed product. At a level of 0.005% of the weight of meat they were able to show excellent protection over storage periods up to twelve months.

3. Use of Antioxidants for Color Protection

The fact that an antioxidant may be successful in protecting the fat from rancidity does not mean that it will also retard oxidative discoloration. There is no reason to suppose that any of the phenolic antioxidants will have a beneficial effect on color. The polyhydroxy phenols do not reduce methemoglobin; in fact, their oxidation products, the quinones, catalyze the oxidation of hemoglobin to methemoglobin (Fishberg, 1948). Wiesman and Ziemba (1946) found that the addition of NDGA to frozen pork sausage actually made the sausage look worse. The author has frequently observed increased methemoglobin formation in frozen fresh meats treated with phenolic antioxidants, even though the antioxidants may have been giving protection against rancidity. Kraft and Wanderstock (1950), on the other hand, found that NDGA dissolved in coconut oil and brushed on the meat surface, protected the surface color of round steaks exposed to light. However, the protection obtained was erratic and no information was given on the effect of the oil carrier alone.

The only antioxidant which has shown any great promise in the protection of meat color is ascorbic acid (Bauernfeind, 1953). This compound reduces methemoglobin and metmyoglobin and has been claimed as a color-stabilizing agent in fresh ground meats (Coleman *et al.*, 1951). Its use in fresh meats seems to be limited to the refrigerated product. At higher temperatures or in the freezer it can accelerate oxidation of hemo-

globin (Watts and Lehmann, 1952a, b). On the other hand, in the presence of nitrite, ascorbic acid accelerates methemoglobin reduction at all temperatures and probably reduces nitrite to nitric oxide (Lugg, 1950), although little published information is available on this step. It can develop cured meat color under conditions where color fixation would otherwise be incomplete and also protect cured meat surfaces from fading when exposed to oxygen.

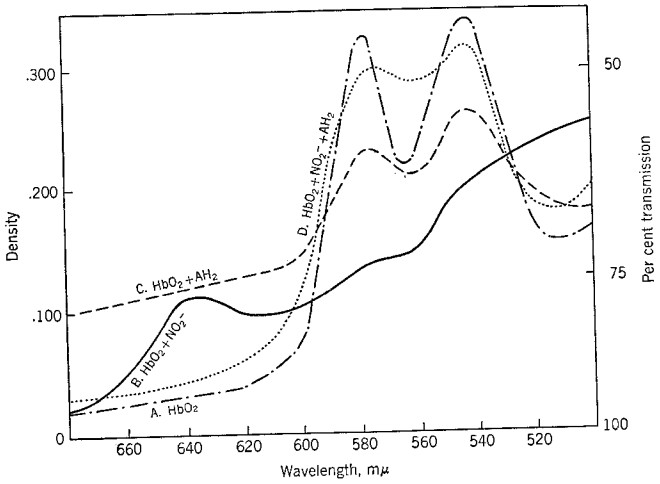

FIG. 5. Changes in the absorption spectrum of oxyhemoglobin brought about by ascorbic acid (Riedesel and Watts, 1952). All solutions except the original oxyhemoglobin were stored for 3 hours at 45° C. (113° F.) before reading.

A. Oxyhemoglobin alone.

B. Oxyhemoglobin plus 0.02% nitrite. This is the typical absorption spectrum of methemoglobin. Solution is brown in color.

C. Oxyhemoglobin plus 0.1% ascorbic acid. Solution greenish brown. A mixture of methemoglobin and choleglobin.

D. Oxyhemoglobin plus 0.02% nitrite and 0.1% ascorbic acid. Typical absorption spectrum of nitric oxide hemoglobin. Bright red.

Figure 5 shows the absorption spectra obtained when ascorbic acid and nitrite, individually and together, were added to aliquots of an oxyhemoglobin solution at 45° C. (113° F.). Nitrite alone converted the oxyhemoglobin immediately to methemoglobin. Ascorbic acid alone gave a mixture of methemoglobin and choleglobin. Nitrite and ascorbic acid, added together, gave the typical absorption spectrum of nitric oxide hemoglobin.

In view of its usefulness in preserving meat color the behavior of ascorbic acid with tissue fats becomes of particular interest. Unfortunately, ascorbic acid (Abramson, 1949) and its homologs (Table VI) can

accelerate oxidation when br
tocopherol. Ascorbic acid it
introduced into aqueous fat sy
whereas the fat-soluble ascorb
1945). Dehydroascorbic acid a
acid. The mechanism of accele
not clear.

The Effect of Ascorbic Acid and Re

Ascorbyl compound
Control
Ascorbic acid
d-Isoascorbic acid
Ascorbyl palmitate
Dehydroascorbic acid

* Lehmann and Watts (1952).
- As indicated by half bleaching of carotene.

Retardation rather than acce
tocopherol or other phenolic a
high (Krukovsky, 1949; Watts a
such as ethylenediaminetetraace
Watts, 1951), which have in com
are added with the ascorbic acid. P
ascorbic acid will be evidenced late
suggesting that the inhibitor is a
position of the ascorbic acid.

When added to meats, ascorbi
of meat and inhibits it in others.
show an acceleration over the co
inhibition after a longer period. It is
into the meat along with a suitable
inhibit oxidation. The difficulties
phenolic antioxidants have already
ascorbic acid with certain artificia
protection in frozen pork produc
example, the following peroxide
sausage after six months storage:

Control.....
0.1% Ascorbic acid.....
0.02% Commercial liquid sm
Ascorbic acid and smoke pre

accelerate oxidation when brought into contact with animal fats low in tocopherol. Ascorbic acid itself brings about greater oxidation when introduced into aqueous fat systems (Scarborough and Watts, 1949), whereas the fat-soluble ascorbyl palmitate oxidizes fat alone (Nagy *et al.*, 1945). Dehydroascorbic acid and *d*-isoascorbic acid behave like ascorbic acid. The mechanism of accelerated fat oxidation by these compounds is not clear.

TABLE VI

The Effect of Ascorbic Acid and Related Compounds on the Oxidation of Lard*

Ascorbyl compound	Days to turn rancid† at 45° C.	
	Plain lard	Lard in contact with aqueous solution, pH 5.8
Control	4.5	5.0
Ascorbic acid	5.5	0.50
d-Isoascorbic acid	5.0	0.50
Ascorbyl palmitate	1.5	2.5
Dehydroascorbic acid	5.0	0.70

* Lehmann and Watts (1952).
† As indicated by half bleaching of carotene.

Retardation rather than acceleration of rancidity occurs if the level of tocopherol or other phenolic antioxidant in the fat is raised sufficiently high (Krukovsky, 1949; Watts and Wong, 1951) or if various compounds such as ethylenediaminetetraacetic acid or polyphosphates (Lehmann and Watts, 1951), which have in common the ability to complex metal ions, are added with the ascorbic acid. Frequently the antioxidant effect of the ascorbic acid will be evidenced later after an initial period of acceleration, suggesting that the inhibitor is an intermediate in the oxidative decomposition of the ascorbic acid.

When added to meats, ascorbic acid accelerates rancidity in some lots of meat and inhibits it in others. Even in the same lot of meat it may show an acceleration over the control after a short storage period but inhibition after a longer period. It is probable that if it could be introduced into the meat along with a suitable phenolic antioxidant, it would always inhibit oxidation. The difficulties of effecting good distribution of the phenolic antioxidants have already been discussed. The combination of ascorbic acid with certain artificial smokes has consistently given good protection in frozen pork products (Watts and Faulkner, 1954). For example, the following peroxide values were obtained in frozen pork sausage after six months storage:

Control	38.1
0.1% Ascorbic acid	70.5
0.02% Commercial liquid smoke preparation	20.0
Ascorbic acid and smoke preparation	5.9

When antioxidants are introduced into meats with the aid of special solvents or emulsifying agents, it is obviously necessary to test the added dispersing agent not only for toxicity but also for effect on rancidity and discoloration. Propylene glycol, for example, has been widely used in experimental studies as a vehicle for introducing antioxidants into meats, since it will dissolve both fat-soluble phenolic substances and also water-soluble synergists. It is the solvent employed in several commercial antioxidant mixtures (Bentz *et al.*, 1952). Klose *et al.* (1952) point out that some commercial samples of propylene glycol accelerate rancidity. It may also accelerate methemoglobin formation in frozen raw (but not cured) meats (Watts and Faulkner, 1953). Furthermore, solution of antioxidants in a small amount of propylene glycol does not insure their uniform distribution in ground meats or curing brines, since dilution of the propylene glycol by the curing brine or muscle juice may cause separation of the less soluble antioxidants unless emulsifying agents are used. Further work on suitable carriers for antioxidants is badly needed.

VI. Effect of Various Meat Constituents and Additives on Rancidity and Discoloration

1. Changes in pH

The pH of normal meat varies from approximately 5.2 to 6.6, owing largely to differences in amount of glycogen available for transformation into lactic acid at the time of slaughter. Factors influencing the glycogen content and pH have been reviewed by Bate-Smith (1949). Animals which have been rested and well fed before slaughter have larger amounts of glycogen stored in the muscle and consequently produce meat of a lower pH.

The optimum pH for meat depends on the purpose for which it is intended. Fresh meat should be at the upper end of the normal pH range if it is to be preserved by freezing, since the reaction between myoglobin and unsaturated fat is inhibited in this range (see table V) and both discoloration and also fat oxidation are retarded. In addition, drip losses are minimized (Sair and Cook, 1938).

Lower pH values also directly accelerate oxidation of fresh meat pigments in the absence of fat. Brooks (1938), Greenwood *et al.* (1940), Watts and Lehmann (1952a), and others have observed the more rapid formation of methemoglobin at lower pH values. Winkler (1939a), studying the relation between pH and light reflectance with several types of meat (pork, beef, and mutton) obtained optimum pink color at a pH of approximately 6. In large cuts of meat, muscles which normally have the

highest pH are those which fade least
1948).

On the other hand, with pork intende
ment, a low pH seems to be preferred. A
is low and penetration of curing salts i
sequently the meat is more subject to
Ingram, 1948; Gibbons and Rose, 195
globin solutions and in comminuted m
curing salts is not a serious problem.
Watts and Lehmann, 1952a, b). Attem
range of normal meat by use of acidifie
cessful cured products, owing to loss o
berg and Miller, 1943; Ingram, 1949b .

There are advantages to the use of hi
meats provided that distribution of the
the color developed by appropriate hea
1940) found that in solutions of pure, pre
oxidation to methemoglobin was less rap
the pH had to be raised above the norm
protection. Brissey (1952) recommends
phates to the curing brines of hams to
elevation of the pH during cure increase
following cooking period. Extensive bac
laboratories showed that under these
no detrimental effect on the keeping qua

2. Salts

No common meat additive has a mo
effect on oxidative changes than sodium c
of salt on rancidity has been widely noted
both fresh and cured meats. Lea (1939
White (1941b) and Gaddis (1952) found t
process accelerated rancidity in bacon (Fig
observed rapid development of rancidity a
in salted fresh pork (Dubois and Tressler
1946; Watts and Peng, 1947a; Watts and

Hills and Conochie (1946), in attempti
effect of salt in butter, proposed a general th
oxidations based on a reaction between fa
and chloride ions, resulting in the formatio

$$R—O—O—H + 2Cl^- + 2H^+ — C$$

highest pH are those which fade least on freezer storage (Watts *et al.*, 1948).

On the other hand, with pork intended for conventional curing treatment, a low pH seems to be preferred. At elevated pH values conductivity is low and penetration of curing salts into the muscle is impeded; consequently the meat is more subject to bacterial spoilage (Callow, 1947; Ingram, 1948; Gibbons and Rose, 1950). Even in homogenous hemoglobin solutions and in comminuted meats where distribution of the curing salts is not a serious problem, low pH accelerates color fixation (Watts and Lehmann, 1952a, b). Attempts to lower the pH beyond the range of normal meat by use of acidified brines have not produced successful cured products, owing to loss of nitric oxide from solution (Duisberg and Miller, 1943; Ingram, 1949b).

There are advantages to the use of higher pH values even with cured meats provided that distribution of the curing salts can be attained and the color developed by appropriate heat treatment. Urbain and Jensen (1940) found that in solutions of pure, preformed nitric oxide hemoglobin, oxidation to methemoglobin was less rapid at high pH values, although the pH had to be raised above the normal limits for meat to get much protection. Brissey (1952) recommends the addition of alkaline phosphates to the curing brines of hams to be sold as "boiled" ham, since elevation of the pH during cure increased retention of juice during the following cooking period. Extensive bacteriological tests in the Swift & Co. laboratories showed that under these conditions the elevated pH had no detrimental effect on the keeping quality of the ham (Jensen, 1953).

2. Salts

No common meat additive has a more profound or more puzzling effect on oxidative changes than sodium chloride. The accelerating effect of salt on rancidity has been widely noted in a variety of foods, including both fresh and cured meats. Lea (1939) has reviewed the earlier work. White (1941b) and Gaddis (1952) found that the salt used in the curing process accelerated rancidity in bacon (Fig. 6). A number of workers have observed rapid development of rancidity and accompanying discoloration in salted fresh pork (Dubois and Tressler, 1943; Wiesman and Ziemba, 1946; Watts and Peng, 1947a; Watts and Lehmann, 1952b).

Hills and Conochie (1946), in attempting to explain the accelerating effect of salt in butter, proposed a general theory for the mechanism of such oxidations based on a reaction between fat-hydroperoxide and hydrogen and chloride ions, resulting in the formation of chlorine;

$$R—O—O—H + 2Cl^- + 2H^+ \rightarrow Cl_2 + H_2O + R—OH$$

The free chlorine resulting from the reaction then brings about further oxidation of the fat. This is the only theory so far proposed for the *fat*-peroxidizing effect of salt.

The accelerating effect of salt on rancidity is evidenced only when conditions are such that the salt is brought into contact with the fat over a wide surface. Since salt is not adsorbed at the interface between fat and

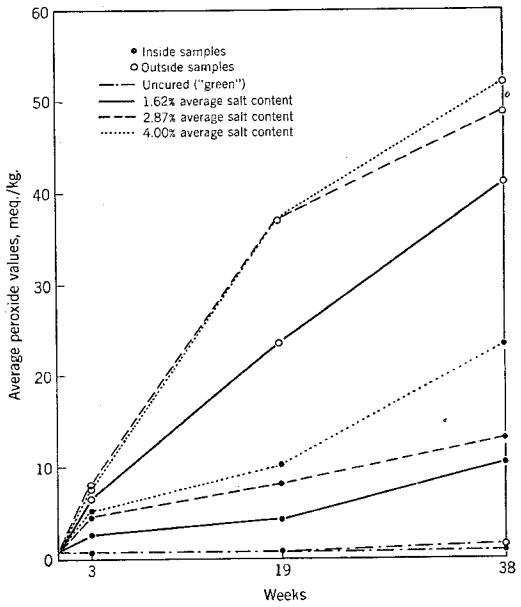

FIG. 6. Effect of salt on oxidation of bacon fat (Gaddis, 1952). Peroxide formation in outside 4 mm. and inside fat from bacon containing different amounts of salt during 3 weeks of curing at 38° F. (3.3° C.) and subsequent freezer storage at 0° F. (−17.8° C.).

water, dilute salt solutions brought into contact with unsaturated fat may actually retard oxidation (Chang and Watts, 1950). However, when these same solutions are partially dried so that the salt is thrown out of solution as a thin surface film, acceleration of rancidity is extremely rapid. This may be an important factor in the rancidification of frozen and dehydrated meats containing salt.

In addition to its effect on fat oxidation, salt also accelerates the oxidation of hemoglobin and myoglobin to the ferric form, whether or not fat is present. Coleman (1951) has summarized the rather extensive literature on this point.

In cured meats, salt, like the hydrogen ion, apparently has just the opposite effect, *i.e.*, improves the cured color. Woodcock and White

(1943) observed this protective effe
Lehmann (1952a, b) found that in the
methemoglobin formation, both in her
in the presence of nitrite and ascorbi
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Like hydrogen ions, salt has a num
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other curing salts.

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3. Me

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catalyst for dry fats (Lea, 1946b).

Recent work on this subject has be
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their catalytic activity. Many of the we
are metal complexing agents. Dutton
effectiveness of various polycarboxyli
improving the stability of soybean oi
found to counteract the effect of pro-ox
phosphates (Watts, 1950; Lehmann a
diaminetetraacetic acid (Trevor, 1949
been suggested as antioxidants for fats i
metal sequestering agents. The phytat

(1943) observed this protective effect on Wiltshire bacon. Watts and Lehmann (1952a, b) found that in the absence of nitrite, salt accelerated methemoglobin formation, both in hemoglobin solutions and in meat, but in the presence of nitrite and ascorbic acid, it accelerated the formation of the bright red cured meat pigment. Salt, like acid, can bring about a partial denaturation of globin. This would account for its opposite effect on fresh and cured meat pigments.

Like hydrogen ions, salt has a number of other important functions in cured meat which are outside the scope of this article. Callow (1947) has summarized earlier studies on swelling and texture changes of muscle tissue in salt. Ingram (1949a) and Hankins *et al.* (1950) discuss the salty flavor in bacon. Bulman and Ayres (1952) have contributed to the already extensive literature on the preservative effect of sodium chloride and other curing salts.

The nitrate and nitrite used in cured meats appear to have little effect on rancidity in the small concentrations in which they are used and at pH values within the normal range of meat (Lea, 1939; Watts and Lehmann, 1952b).

3. Metals

The earlier literature on the effect of metals on rancidity in fats has been well covered by Lea (1939). The accelerating effect of copper and iron on oxidation of fats and fat-containing foods is well established by a wealth of experimental evidence. Other metals, particularly vanadium, cobalt, and manganese, may also be strong pro-oxidants but are less likely to contaminate foods. These metals can accelerate rancidity either in the form of the solid metal in contact with the fat or as water- or fat-soluble salts in heterogenous systems. Aluminum, tin, and zinc have little catalytic activity under most conditions, although tin can act as a surface catalyst for dry fats (Lea, 1946b).

Recent work on this subject has been concerned mainly with the use in fats or fat-containing foods of various chemicals which can form precipitates or soluble nonionized complexes with metals and so diminish their catalytic activity. Many of the well-known synergistic antioxidants are metal complexing agents. Dutton *et al.* (1948) have studied the effectiveness of various polycarboxylic acids and polyhydric alcohols on improving the stability of soybean oil. Citric acid and sorbitol were found to counteract the effect of pro-oxidant metals. More recently polyphosphates (Watts, 1950; Lehmann and Watts, 1951) and ethylenediaminetetraacetic acid (Trevor, 1949; Watts and Wong, 1951) have been suggested as antioxidants for fats in heterogenous systems. Both are metal sequestering agents. The phytates, which form both soluble and

insoluble salts with metals (Cohee and Steffen, 1949) did not show antioxidant activity in aqueous fat systems under the same conditions (Lehmann and Watts, 1952).

It is not necessarily true that formation of nonionized complexes will reduce the catalytic activity of metals on fat oxidation. The great increase in fat oxidase activity of iron porphyrins over inorganic iron has already been discussed. Still more active catalysts are formed by complexing iron with phenanthroline (Simon *et al.*, 1944).

The part played by metals in oxidative changes of meats is virtually an unexplored field. Published information on metal contamination of meats or on the effect of added metals or metal sequestering agents in meat is very limited. Chang and Watts (1949) found that citric acid and several polyphosphates which act as synergistic antioxidants in aqueous fat systems did not retard fat oxidation when catalyzed by hemoglobin, but were effective after the hemoglobin was coagulated by heat.

In the above experiments, the polyphosphates had an adverse effect on color, accelerating oxidation of oxyhemoglobin. On the other hand, Hall (1950) recommended the use of polyphosphates for preserving the color in frankfurters. Weiss *et al.* (1953) found that, whereas copper and iron accelerated the oxidation of oxyhemoglobin, these same metals as well as zinc catalyzed the reduction of methemoglobin and color fixation by ascorbic acid in the presence of nitrite. The addition of a metal sequestering agent, ethylenediaminetetraacetic acid, interfered with formation of nitric oxide hemoglobin.

4. Smoke

It has been known for many years that smoking of flesh foods increases their resistance to rancidity. Lea (1933), White (1941b, 1944), Smith *et al.* (1945), Grant and White (1949), Gaddis (1952), and a number of others have demonstrated the antioxidant effect of the smoke treatment by appropriate chemical tests on the fat exposed to the smoke. Jensen (1945) has reviewed the literature on the smoking process, the chemical composition of wood smoke, and its penetration into meat. Many classes of compounds have been found, including unidentified phenolic substances. Presumably the antioxidants belong to this latter class, although this is by no means certain. The concentration of smoke constituents is highest on the outside of a smoked ham; very little penetration of the smoke to the center tissues takes place. Thus, while uncut hams or sides of bacon are protected from oxidative changes, slices from the smoked meat may be unprotected over much of their surface area. However, Gaddis (1952) observed some protection of the inner portion of sides of bacon as well as the outer four mm. strip (Fig. 6). Johnson

(1950) recommends the smoki
uniform and efficient distributi

Several artificial smoke fla
the market. These differ in th
quality and intensity of the sm
thermal decomposition of hard
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found to vary from no protecti
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To date, no imitation smoke
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distributed throughout the meat.

There is little mention in the
ingredients on color of meat. H
heat treatment is involved, is im
Table IV). The phenolic consti
inhibitors of fat oxidation, caus
storage.

Many natural spices and other
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ground nut oil with chilies, cinna
pepper, cloves, and mace. Chipaul
spices and found that most of the
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natural spices were extracted, fra
did not have a strong antioxidant

Dubois and Tressler (1943) an
keeping time of frozen pork sausa
pepper were good antioxidants. I

(1950) recommends the smoking of individual slices of bacon for more uniform and efficient distribution of the smoke.

Several artificial smoke flavors or "liquid smokes" are available on the market. These differ in their method of manufacture and in the quality and intensity of the smoke flavor, although all are derived from thermal decomposition of hardwood. The effect of several of these preparations on fat oxidation has been tested (Watts and Faulkner, 1954) and found to vary from no protection or even a slight pro-oxidant effect at one extreme to a strong antioxidant effect in concentrations of a few hundredths per cent of the commercial preparation at the other. The antioxidant activity of the latter preparation thus approaches that of the pure phenolic inhibitors. Discolorations with iron salts, such as occur with gallates and other polyhydroxy phenols, do not take place with these liquid smokes.

To date, no imitation smoke flavor has been permitted by the Bureau of Animal Industry in meat products being shipped across state boundaries. Although these imitation smokes can certainly not perform the many other functions of the conventional smoking process, such as partial drying of the meat and surface gloss, there would seem to be some advantage in their use as antioxidants as well as flavor constituents in curing brines, particularly since, unlike many of the best phenolic inhibitors, they can be dispersed in the brine and thus more uniformly distributed throughout the meat.

There is little mention in the literature of specific effects of smoke ingredients on color of meat. However, the smoking process itself, since heat treatment is involved, is important in developing cured meat color (Table IV). The phenolic constituents of smoke, like other phenolic inhibitors of fat oxidation, cause browning of fresh meat in freezer storage.

5. Spices

Many natural spices and other condiments have an antioxidant effect when added to fats. Sethi and Aggarwal (1950) obtained protection of ground nut oil with chilies, cinnamon, ginger, turmeric, nutmeg, black pepper, cloves, and mace. Chipault *et al.* (1952) tested thirty-two ground spices and found that most of them had antioxidant properties with lard. Rosemary and sage were particularly effective. However, when the natural spices were extracted, fractions containing the odor components did not have a strong antioxidant effect.

Dubois and Tressler (1943) and Atkinson *et al.* (1947) extended the keeping time of frozen pork sausage by the use of spices. Sage and black pepper were good antioxidants. It should be noted, however, that the

antioxidant effect of the combined spices is not usually as great as the prooxidant effect of the salt, so that untreated pork usually keeps better than seasoned sausage.

VII. Physical Factors Affecting Oxidative Changes

1. Oxygen Tension

Niell and Hastings (1925) demonstrated more rapid conversion of hemoglobin to methemoglobin at intermediate rather than at very high or very low oxygen tensions. This was true not only for the spontaneous oxidation of laked blood corpuscles but also for the oxidation of hemoglobin catalyzed by unsaturated fats.

Brooks (1929, 1935, 1936, 1938) has studied extensively the penetration of oxygen into muscle tissues and the effect of such penetration on meat color. In early experiments Brooks (1929) devised a simple technique for following oxygen penetration and pigment oxidation. Slices of tissue were placed on a glass slide between thin rods of glass and compressed with a cover glass. A Zeiss microspectroscope allowed examination of tissue pigments at different distances from the air tissue interface. A steady state was achieved after oxygen had penetrated to a depth of approximately 2 mm. Further penetration occurred only after long standing and was attributed to slow decrease in oxygen consumption by the tissue. The interior of the slice remained completely reduced. Methemoglobin formed only in the thin region of oxygen penetration and most rapidly in the inner part of this region. The outer part was largely oxyhemoglobin. Freezing and thawing the tissue had no effect on the rate or depth of penetration but did accelerate methemoglobin formation in the region of oxygen penetration.

Unlike hemoglobin and myoglobin, the corresponding nitric oxide derivatives do not show an intermediate optimum oxygen pressure for methemoglobin formation but oxidize more rapidly the higher the oxygen pressure (Brooks, 1935; Urbain and Jensen, 1940). This might be expected, since dissociation of these derivatives would not be increased by lowering the oxygen tension.

2. Light

Light accelerates all oxidative changes in meats, provided, of course, that oxygen is available. Discolorations caused by exposure to light have become a particularly serious problem because of modern methods of merchandising which require exposure of retail cuts in lighted display cases. Cured meats are much more susceptible to light discoloration than fresh (Ramsbottom *et al.*, 1951; Urbain and Ramsbottom, 1948). Appar-

ently light can bring about a dis
similar to its well-known effect on c
case lighting does not significantly
three days, but fading of cured, sm
able in an hour under the same co
(incandescent, tungsten-filament, a
fading for the same time of exposure
in the frozen state does not reduce
violet light appears to have no great
intensity on oxidation of the cured
fresh meat pigments, which are n
when treated with ultraviolet light
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its oxidizing effect on hemoglobin a

The important facts concerning t
were for the most part established
literature has been critically evalua
reviewed in detail here. Some of th
drawn from this work are as follow
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For example, beef kidney fat which w
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increases the rate of peroxide formati

The use of ultraviolet light to reta
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Volz *et al.* (1949) found that skinned
more than two days exposed to ultra
rancid during subsequent freezer st
shading the carcasses or by leaving
Brady *et al.* (1949) found no direct
pork and peroxide formation after fre

3. Tempe

As might be expected, the oxidati
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reviewed the earlier literature on temp

ently light can bring about a dissociation of nitric oxide hemoglobin similar to its well-known effect on carbon monoxide hemoglobin. Display case lighting does not significantly discolor fresh meats in periods up to three days, but fading of cured, smoked, and table-ready meat is noticeable in an hour under the same conditions. The several kinds of lighting (incandescent, tungsten-filament, and fluorescent) all bring about equal fading for the same time of exposure and light intensity. Display of meats in the frozen state does not reduce their susceptibility to fading. Ultraviolet light appears to have no greater effect than visible light of the same intensity on oxidation of the cured meat pigments. On the other hand, fresh meat pigments, which are not affected by visible light, discolor when treated with ultraviolet light. Ultraviolet light is known to bring about protein denaturation (Haurowitz, 1950). This would account for its oxidizing effect on hemoglobin and myoglobin.

The important facts concerning the effect of light on oxidation of fats were for the most part established many years ago. The voluminous literature has been critically evaluated by Lea (1939) and will not be reviewed in detail here. Some of the more important conclusions to be drawn from this work are as follows: Shorter wavelengths of light are more effective in causing oxidation than longer; ultraviolet light is an extremely potent accelerator of rancidity even in relatively stable fats. For example, beef kidney fat which was not rancid after 1200 hours when stored in the dark showed perceptible rancidity in 10 minutes when placed in strong sunlight (containing a high proportion of ultraviolet rays). The most noticeable effect of light is the elimination of the induction period, although the rate of subsequent oxygen uptake can also be accelerated by light of high intensity. Illumination of fats not only causes more rapid peroxidation during the period when the fat is exposed to light, but also increases the rate of peroxide formation after the light is removed.

The use of ultraviolet light to retard growth of microorganisms in cold storage rooms where meat is hung can cause rancidity, especially in pork. Volz *et al.* (1949) found that skinned pork (but not beef) carcasses held more than two days exposed to ultraviolet light prior to freezing turned rancid during subsequent freezer storage. This could be prevented by shading the carcasses or by leaving on the skins. On the other hand Brady *et al.* (1949) found no direct relationship between irradiation of pork and peroxide formation after freezing storage.

3. Temperature

As might be expected, the oxidation rate of both heme pigments and fat of meat is accelerated with increasing temperature. Lea (1939) has reviewed the earlier literature on temperature coefficients of fat oxidation.

In general, the oxidation rate of pure dry oils was approximately doubled by a 10° C. (18° F.) rise in temperature in the absence of catalysts. In the presence of light or metal catalysts the coefficient was much smaller. There is no published information on the temperature coefficient of the coupled reaction between hemoglobin and unsaturated fat. Numerous more recent studies on frozen meats and poultry have emphasized the importance of low storage temperatures in retarding rancidity (Cook and White, 1939, 1941; Ramsbottom, 1947; Atkinson *et al.*, 1947; Hall *et al.*, 1949; Klose *et al.*, 1950; Palmer *et al.*, 1953).

At the other extreme, high (cooking) temperatures undoubtedly accelerate the oxidation of meat fats, but since peroxides and the various aldehydes used to measure rancidity are unstable at high temperatures, results are likely to be erratic. Chang and Watts (1952) found considerable peroxidation of the fat in meat and drippings when large cuts of pork, beef, lamb, and poultry were roasted. On the other hand, the peroxide values of body fat and drippings from frozen cuts of meat, originally high, fell during the cooking period. Hanson *et al.* (1950) found roasting to be much inferior to cooking in water as a preliminary treatment for frozen poultry.

Although discolorations often accompany rancidity in frozen storage studies such as those listed above, the lack of objective methods for following such changes make it much more difficult to obtain even semi-quantitative data on relative rates of discoloration at various temperatures. Cook and White (1941) measured reflected light from frozen pork stored at various temperatures. Although methemoglobin did not form at the lower storage temperatures the meat was darker, possibly owing to desiccation. Urbain and Jensen (1940) have pointed out the high temperature coefficient of nitric oxide hemoglobin oxidation. Solutions which were completely oxidized in less than a day at 37° C. (99° F.) required thirteen days at 10° C. (50° F.). On the other hand, Ramsbottom *et al.* (1951) observed that frozen cured meats exposed to light faded almost as rapidly as refrigerated samples. It is possible that hemoglobin oxidation, like fat oxidation, is less sensitive to temperature changes when light or catalysts are present, but quantitative data are lacking.

4. Packaging Problems

The main considerations in the packaging of cured meat products are the exclusion of oxygen and light. Any type of packaging which reduces contact with oxygen retards both rancidity and discoloration. Whenever tried, vacuum and gas packing have resulted in improved keeping qualities. Storage of meat in atmospheres containing carbon dioxide has been found in a number of studies to retard bacterial action as well as

oxidative changes (Brooks, 1933; Kraft and Ayres, 1952). Ability ... gas is, of course, an importan... found that frankfurters did not ... dioxide had been lost from the p... and Ramsbottom *et al.* (1951 ... in packaged cured meats.

Light exclusion interferes ... Transparent wrappings can be ... and shorter wavelengths of ligh... 1939), but since fading of cured ... the only wrappings which are ... deeply colored that they create se... 1948; Ramsbottom *et al.*, 1951 ... only in the presence of oxygen. ... detrimental effect of light.

With refrigerated fresh meats ... the fact that the purplish red of ... the oxygenated compound. Als... oxidation to methemoglobin, an... packaging further accelerates th... generally been found preferable ... passage of oxygen and to limit ... centrations (Kraft and Ayres, 195... optimum for preventing rancidity.

In freezing preservation of ... been placed on use of packages ... moisture. Various fresh meats rem... longer when packed in nitrogen ... Hiner *et al.*, 1951). Numerous ... desiccation or "freezer burn" wit... Winkler, 1939b; Cook and W... many others). In most of these ... between the effect of desiccation a... berg *et al.* (1949) separated these t... levels with and without a desicca... affected by the desiccant, but p... oxygen levels.

Impermeable wrappings can di... or fluctuating freezer temperatur... significant differences in frozen gr... compared to fluctuating temperat...

oxidative changes (Brooks, 1933; Lea, 1939; Ogilvy and Ayres, 1951a, b; Kraft and Ayres, 1952). Ability of the packaging material to retain the gas is, of course, an important consideration. Kraft and Ayres (1952) found that frankfurters did not spoil until some time after the carbon dioxide had been lost from the package. Urbain and Ramsbottom (1948) and Ramsbottom *et al.* (1951), stress the desirability of excluding oxygen in packaged cured meats.

Light exclusion interferes with the transparency of the package. Transparent wrappings can be obtained which eliminate the ultraviolet and shorter wavelengths of light mainly responsible for rancidity (Lea, 1939), but since fading of cured meats is accelerated by visible light rays, the only wrappings which are very effective in retarding fading are so deeply colored that they create sales resistance (Urbain and Ramsbottom, 1948; Ramsbottom *et al.*, 1951). Since light accelerates oxidative changes only in the presence of oxygen, vacuum or gas packing can eliminate the detrimental effect of light.

With refrigerated fresh meats the problem is further complicated by the fact that the purplish red of reduced hemoglobin is less desirable than the oxygenated compound. Also lowered oxygen tension accelerates oxidation to methemoglobin, and lowered pH caused by carbon dioxide packaging further accelerates the oxidation. For these reasons it has generally been found preferable to use wrappings which allow some passage of oxygen and to limit the use of carbon dioxide to lower concentrations (Kraft and Ayres, 1952), even though these conditions are not optimum for preventing rancidity and other types of spoilage.

In freezing preservation of both cured and fresh meats emphasis has been placed on use of packages which are impermeable to oxygen and moisture. Various fresh meats remained palatable in freezer storage much longer when packed in nitrogen (Steinberg *et al.*, 1949) or vacuum packed Hiner *et al.*, 1951). Numerous workers have observed a correlation of desiccation or "freezer burn" with oxidative rancidity and discoloration Winkler, 1939b; Cook and White, 1939; Ramsbottom, 1947; and many others). In most of these studies it is impossible to distinguish between the effect of desiccation as such and the effect of oxidation. Steinberg *et al.* (1949) separated these two factors by storing at different oxygen levels with and without a desiccant. In this study, color was adversely affected by the desiccant, but palatability scores depended only upon oxygen levels.

Impermeable wrappings can do much to offset adverse effects of high or fluctuating freezer temperatures. Winter *et al.* (1952) obtained highly significant differences in frozen ground meat stored at $-18°$ C. (0° F.) as compared to fluctuating temperatures between this and $-10°$ C. (13° F.)

when waxed freezer paper wrappings were used, but the fluctuating temperatures had no adverse effect when the meat was wrapped in laminated aluminum foil. Hanson *et al.* (1950) found the type of package to be of greater importance than the storage temperature for retention of quality in precooked frozen poultry.

VIII. Summary

Measures for the control of oxidative rancidity in meats can begin with the feeding of the meat animals. Rancidity of the fat *in situ* is influenced by the characteristics of the fat itself and by the aqueous medium with which it is intimately associated.

The inherent characteristics of the fat which have an effect on rancidity have been established. They are: (1) the fatty acid composition, particularly the number of active methylene groups between unsaturated carbon atoms which occur in fatty acids having two or more double bonds; and (2) the amount of natural antioxidant, specifically *alpha* tocopherol, stored in the fat.

Within any one species of meat animal, these factors are determined largely by ration. By greatly reducing the more highly unsaturated fats in the ration, the body fat of hogs and poultry is rendered much less susceptible to rancidity. However, unsaturated fats are present in many of the important stock feeds such as soybeans, alfalfa, and fish meal. While it is not possible or desirable to eliminate such feeds, it might well be feasible to select animals which have not received sources of highly unsaturated fat when it is expected that their meat will be stored for a period of time under conditions where rancidity might be a problem.

At present economic factors also preclude the feeding of tocophorol concentrates to meat animals. Large doses of this vitamin in the feed do increase fat stability, but most of it is excreted. Only a small fraction of that fed is absorbed from the digestive tract and stored in the fat. It is possible that deposition of either tocopherol or fatty acids or both may be influenced by the feeding of substances which can affect fat metabolism, but such studies are too few and in too early a stage to warrant any conclusions at present.

Accelerators and inhibitors of rancidity in the aqueous muscle juice are undoubtedly also of importance, but research has not advanced sufficiently to illuminate more than the fringes of the field. Hemoglobin and myoglobin are known to bring about very rapid oxidation of fat along with their own self-destruction. Thus contact of these pigments with unsaturated fat in the presence of oxygen results in rancidity and discoloration. This reaction is probably of particular importance in ground meats preserved by freezing and in meats in cure, where the heme pig-

ments can dissolve in the
meats where the pigments
meats in the higher range

A number of normal
amino acids, biotin, niacin,
added to pure fats contain
ascorbic acid, there is no in
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protective influence.

Salt, as used in curing
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Fading and discoloratio
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ments can dissolve in the brine. It probably does not occur in cooked meats where the pigments are denatured. The reaction is much slower in meats in the higher range of normal pH.

A number of normal constituents of muscle juice, including various amino acids, biotin, niacin, and ascorbic acid, can inhibit rancidity when added to pure fats containing phenolic inhibitors. With the exception of ascorbic acid, there is no information on the effect of these substances on fats in close contact with an aqueous phase or on fat oxidations catalyzed by hemoglobin. Nor is it known whether the limited amounts of these substances which actually occur in muscle juice, in conjunction with the limited amount of tocopherol naturally present in the fat, can exert a protective influence.

Salt, as used in curing or when added to frozen ground meats, definitely accelerates rancidity. Smoke constituents, including some artificial smokes, and many spices, inhibit it. Newer phenolic inhibitors are very effective in protecting fat even in the presence of an aqueous phase containing hemoglobin and could possibly effect the same protection in meats provided that uniform distribution could be attained in the meat. However, this is difficult, since their solubility is often too limited to allow them to be incorporated readily in ground meats, curing brines, or cooking waters. Various carriers and surface coatings designed to assist in getting the necessary antioxidant distribution have been described but the problem is far from solution.

Fading and discoloration of meats is due to oxidation of the normal pigments hemoglobin and myoglobin of fresh meat or their nitric oxide derivatives in cured meat, all ferrous heme compounds. The oxidation products are the same for fresh and cured meat pigments. They consist usually of the ferric pigments, methemoglobin and metmyoglobin, brown in color, and less frequently green or faded decomposition products of the porphyrin ring.

Although both fresh and cured meat pigments oxidize along the same pathways and to the same brown or green end products, changes in the physical and chemical environment affect the oxidation of these pigments very differently. Some of these differences between the two pigments may be traced to variations in their dissociation. The available evidence indicates that both the oxymyoglobin of fresh meat and the nitric oxide myoglobin of cured meats must dissociate to myoglobin before oxidation to the brown ferric metmyoglobin takes place. The dissociation of oxyhemoglobin increases with decreasing oxygen tension. Fresh meat pigments therefore turn brown more readily at oxygen tensions considerably below that of air at atmospheric pressure. In contrast, dissociation of nitric oxide hemoglobin does not increase at lower oxygen tension, so that

rate of oxidation of cured meat pigment is progressively increased with increasing oxygen supply. Again, visible light fades cured meats more rapidly than fresh, probably because of a dissociating effect on nitric oxide hemoglobin similar to the known effect of light on carbon monoxide hemoglobin.

Furthermore, any treatment which tends to denature the globin, even partially or reversibly, serves to weaken the bonds between heme and globin. Without these stabilizing bonds to native globin, heme loses the ability to combine reversibly with oxygen to form the bright red oxygenated compound and instead is oxidized very rapidly to the brown ferric form. On the other hand, the bright red color of nitric oxide hemoglobin is not lost even by complete and irreversible denaturation of the globin. In fact, globin denaturation seems to accelerate the formation of cured meat pigments.

Thus, as is well known from common experience, the heating of meat to temperatures sufficiently high to denature the globin results in the brown ferric hemochromogen of cooked meat. The heat-denatured nitric oxide hemochromogen, on the other hand, retains the red color of the ferrous compound, and some form of heat treatment is widely practiced in the manufacture of cured meats. Likewise increased acidity, salt, certain metals, freezing, and probably a variety of other environmental conditions accelerate methemoglobin formation in fresh meat pigments, but these factors either have no effect on cured meat pigments or actually improve color fixation.

Direct attack on the porphyrin ring to produce green derivatives can probably occur with any of the heme compounds, but again the optimum conditions for producing such discolorations are not the same for fresh and cured meat pigments. For example, free hydrogen peroxide (usually of bacterial origin) has much less effect on fresh than on cured meat—probably because of the catalase activity of the fresh meat—and hydrogen donors such as ascorbic acid protect nitric oxide myoglobin but accelerate oxidation of oxymyoglobin.

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The Chemistry of the Sugar–St
Food

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C

I. Intro

One of the oldest but as yet most preservation of fruits and dehydrated changes involves treatment in some extensive commercial application. sulfurous acts is no more clear than the sulfurations themselves develop. How ever, at least in the prevention of sugars, either naturally present or re during processing, somehow influen